UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

January 8, 2016

MEMORANDUM

Subject: Efficacy Review for White;
EPA Reg. No. 777-REI;
DB Barcode: D429903

From: Son Nguyen
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

To: Eric Miederhoff RM31
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser
Morris Corporate Center IV
399 Interpace Parkway
Parsippany, NJ 07054-0225

Formulation from the Label:

Active Ingredient(s)	% by wt.
Alkyl dimethyl benzyl ammonium chloride	0.960%
Octyl decyl dimethyl ammonium chloride	0.720%
Dioctyl dimethyl ammonium chloride	0.288%
Didecyl dimethyl ammonium chloride	0.432%
Other Ingredients	97.60%
Total	100.00%

I. BACKGROUND

The product, White (EPA Reg. No. 777-REI), is a new product for use as a laundry sanitizer on soft surfaces. The applicant is requesting to register the product to add claims of effectiveness as a laundry sanitizer against *K. pneumoniae* and *S. aureus*. The studies were conducted at Accuratus Lab Services, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

The data package contained a letter from the applicant to EPA (dated August 18, 2015), EPA form 8570-1 (Application for Pesticide Registration), EPA form 8570-34 (Certification with Respect to Citation Data), EPA Form 8570-35 (Data Matrix), 2 efficacy studies (MRID No. 49684513 and No. 49684514), Confidential Statements of Formula, and the proposed label. A Statement of No Data Confidentiality Claims, Good Laboratory Practice Statement and Quality Assurance Unit Summary were included with the studies.

II. USE DIRECTIONS

The product, White, is a laundry sanitizer designed to be used against bacteria on soft-surfaces. The product may be used to treat soft surfaces including: Baby/kids clothes, bath rug, bedding, blouses, cotton, cotton/polyester, delicates, fabrics, gym/sports clothes, linens, moist/sweaty clothes, nylon, nylon/polyester, pajamas, pants, pet bed, polyester, polyester/spandex, rayon, shirts, socks, swim wear, synthetic fabrics, travel laundry, undergarments. Directions on the proposed label provide the following information regarding preparation and use of the product:

To sanitize: Add to rinse cycle and leave product in (contact) (rinse cycle) for 16 minutes.

For (Standard) (Top Load) Machine(s): Add (150mL) ((X) capful(s) each filled to (level) (line) (X) of the dosing cup) to the fabric softener (compartment) (drawer) (of the washing machine) or directly to your rinse cycle wash.

For (High Efficiency) (Front Load) Machine(s): Add (100mL) ((X) capful(s) each filled to (level) (line) (X) of the dosing cup) to the fabric softener (compartment) (drawer) (of the washing machine) or directly to your rinse cycle wash. (If total amount does not fit, add the remaining amount into the (compartment) (drawer) when rinse cycle wash begins.)

<<ADVISORY STATEMENT>>
Not intended to be mixed with laundry detergents. Use only in rinse cycle.
Can be used on (most) (majority of) washable (fabrics) (materials). (Not intended for use on) (silk) (or) (wool).
Do not use on materials that cannot be washed in (a) washing machine(s).

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sanitizing Laundry Additives:
The effectiveness of laundry sanitizers must be supported by data that show that the product will substantially reduce the numbers of test bacteria on fabric and in laundry water. Laundry additives may either be used as soaking treatments prior to laundering or as treatments added during laundry operations. The label must specify the type of use. Laundry additives may be recommended for household/coin-operated machine use or commercial-industrial-institutional use. The label must specify the type of use. There is a significant difference in the water to fabric ratio between these two uses, which may affect the efficacy of the product. The Agency

recommends a stimulated-use study using the American Society for Testing and Materials (ASTM) Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants (E 2274-03) (Ref. 3) or Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants For Use in High Efficiency Washing Operations (E 2406-04) (Ref. 4). Alternately, an actual in-use study utilizing washing machines may be used. Tests should be conducted with three samples representing three different batches. Each sample should be tested using three cloth swatches for each target microorganism, which are *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Products labeled as being suitable for hospital use must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). The method should be designed to include testing of both the fabric and laundry water (5 mL from the automatic washer, or 0.5 mL from the simulated washing device) in individual wide-mouth jars containing subculture media and neutralizers. The laundry water-to-media volume ratio must not exceed 1:40. The effectiveness of an antimicrobial laundry product may be altered by differences in laundry machine types (top loading vs. high efficiency machines). The water to fabric ratio in common top loading machines is approximately 10:1 (wash volume to fabric weight), while with high efficiency laundry machines, it can be as low as approximately 2.5:1. A minimum average of Results from a quantitative bacteriological assay must be reported. Results must show a bacterial reduction of 99.9% (a 3-$\log_{10}$ reduction) of bacteria over the control count for both fabric and laundry water for each organism tested. The label directions for use of laundry additives should specify the machine cycle in which the product is to be added, as well as water level, temperature, and treatment time. Compatibility of the treatment with other laundry additives should be determined in testing and addressed in labeling, when applicable.

Supplemental Claims:
An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

IV. SYNOPSIS OF SUBMITTED EFFICACY STUDY

1. MRID 49684513 "Standard Test Method for the Evaluation of Laundry Sanitizers" Test Organisms: *Klebsiella pneumoniae* (ATCC 4352) for White, by Jamie Herzan. Study conducted at Accuratus Lab Services. Study completion date – 06/30/2015. Project No. 18622.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). Three batches (Batch 2037-054, Batch 2037-055 and Batch 2037-056) of the product, White, were tested using Accuratus Lab Servives Labs Protocol No. REK01060815.LSAN.1 (copy provided). The product was diluted 1:173 (defined as 1 part test substance+ 172 parts diluent) using 3.0mL of the test substance and 516 mL 200 ppm AOAC synthetic hard water, while the test substance to fabric ratio was 10:1 (weight/weight) for Household Applications. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.14% Lecithin and 1.0% Tween 80 was used as neutralizer subculturing solution. Numerous sets of sterile "exposure chamber" Nalgene jars were filled with 150 ± 0.1 grams of the prepared test substance and equilibrated to 20±1°C. The culture was prepared without an organic soil load. The fabric carrier prepared on 4/24/15 was a plain cotton weave containing 80 x 80 threads/inch and 640 grams of test fabric was added to each 6.4 L volume of scouring solution (3.2 grams Na_2CO_3 and 3.2 grams of Triton X-100 in 6.4 L of deionized water). The fabric carrier prepared on 6/8/15 was a plain cotton weave containing 80 x 80 threads/inch and 355 grams of test fabric

was added to each 3.5 L volume of scouring solution (1.75 grams Na_2CO_3 and 1.75 grams of Triton X-100 in 3.5 L of deionized water). The solution was boiled for 60 minutes. The fabric then was rinsed in boiling water for 5 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air drying for at least a day, the fabric was cut into 5 cm (2 in.) wide strips weighing 15±0.1 gram. Each fabric strip was wrapped around a stainless steel spindle at least 12, but fewer than 13 times. Swatches (1 in. by 1.5 in.) were then cut from the remaining fabric. All carriers were autoclave sterilized and were allowed to cool/dry at room temperature. Three fabric swatch carriers per product lot were inoculated with 30µl of prepared test organism and dried in a 35-37°C incubator at 41% relative humidity for 30 minutes. After drying, up to three fabric carriers were placed individually into the spindle pockets between the 6th and 7th folds of the spindle. The fabric spindles containing the carriers were placed into the Nalgene jars containing the use solution. The jars were placed into a laundrometer device to simulate a tumble-wash at 45-60 RPM for a 15 minute exposure time at 20±1°C. Following completion of the simulated wash, the fabric carriers were aseptically removed and placed individually into jars containing 10 mL of the neutralizer. A 0.50 mL aliquot of the "wash" water was transferred to a vessel containing 9.5 mL of the neutralizer. The subcultures were vortex mixed for approximately 10 seconds and ten-fold serial dilutions were prepared. For *K. pneumonia*, 1.00 mL of the 10^0 and 0.100 mL aliquots of the 10^0 through 10^{-3} dilutions were spread plated in duplicate. All plates were incubated for 48-54 hours at 35-37°C. Following incubation, standard plate count procedure were used to determine the average colony forming unit per carrier and per milliliter of wash water. On 6/20/15, representative test and positive control subcultures showing growth were visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism. Controls included those for culture purity, carrier sterility, numbers control, initial suspension control, and neutralization confirmation. The reported average Colony Forming Units per carrier for the test microorganism is: ***Klebsiella pneumoniae* 4.27 x 10^6.**

Note: There were no protocol changes.

2. **MRID 49684514 "Standard Test Method for the Evaluation of Laundry Sanitizers" Test Organisms: *Staphylococcus aureus* (ATCC 6538) for White, by Jamie Herzan. Study conducted at Accuratus Lab Services. Study completion date – 08/05/2015. Project No. 18839.**

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three batches (Batch 2037-054, Batch 2037-055 and Batch 2037-056) of the product, White, were tested using Accuratus Lab Servives Labs Protocol No. REK01070115.LSAN.1 (copy provided). The product was diluted 1:173 (defined as 1 part test substance+ 172 parts diluent) using 2.0 mL of the test substance and 344.0 mL 200 ppm AOAC synthetic hard water, while the test substance to fabric ratio was 10:1 (weight/weight) for Household Applications. Tryptic Soy Agar with 5% Sheep Blood was used as agar plate medium. Letheen Broth with 0.14% Lecithin and 1.0% Tween 80 was used as neutralizer subculturing solution. Numerous sets of sterile "exposure chamber" Nalgene jars were filled with 150 ± 0.1 grams of the prepared test substance and equilibrated to 20±1°C. The culture was prepared without an organic soil load. The fabric carrier prepared on 4/24/15 was a plain cotton weave containing 80 x 80 threads/inch and 640 grams of test fabric was added to each 6.4 L volume of scouring solution (3.2 grams Na_2CO_3 and 3.2 grams of Triton X-100 in 6.4 L of deionized water). The fabric carrier prepared on 7/10/15 was a plain cotton weave containing 80 x 80 threads/inch and 690 grams of test fabric was added to each 6.9 L volume of scouring solution (3.45 grams Na_2CO_3 and 3.45 grams of Triton X-100 in 6.9 L of deionized water). The solution was boiled for 60

minutes. The fabric then was rinsed in boiling water for 5 minutes and then rinsed in cold water for 5 minutes. During the rinsing procedure, the fabric was mixed in the water to help remove the wetting agent. After air drying for at least a day, the fabric was cut into 5 cm (2 in.) wide strips weighing 15±0.1 gram. Each fabric strip was wrapped around a stainless steel spindle at least 12, but fewer than 13 times. Swatches (1 in. by 1.5 in.) were then cut from the remaining fabric. All carriers were autoclave sterilized and were allowed to cool/dry at room temperature. Three fabric swatch carriers per product lot were inoculated with 30 µl of prepared test organism and dried in a 35-37°C incubator at 42% relative humidity for 25 minutes. After drying, up to three fabric carriers were placed individually into the spindle pockets between the 6th and 7th folds of the spindle. The fabric spindles containing the carriers were placed into the Nalgene jars containing the use solution. The jars were placed into a laundrometer device to simulate a tumble-wash at 45-60 RPM for a 16 minute exposure time at 20±1°C. Following completion of the simulated wash, the fabric carriers were aseptically removed and placed individually into jars containing 10 mL of the neutralizer. A 0.50 mL aliquot of the "wash" water was transferred to a vessel containing 9.5 mL of the neutralizer. The subcultures were vortex mixed for approximately 10 seconds and ten-fold serial dilutions were prepared. For *S. aureus*, 1.00 mL of the 10^0 and 0.100 mL aliquots of the 10^0 through 10^{-3} dilutions were spread plated in duplicate. All plates were incubated for 48-54 hours at 35-37°C. Following incubation, standard plate count procedure were used to determine the average colony forming unit per carrier and per milliliter of wash water. On 7/23/15, representative test and positive control subcultures showing growth were visually examined, Gram stained and biochemically assayed to confirm or rule out the presence of the test organism. Controls included those for culture purity, carrier sterility, numbers control, initial suspension control, and neutralization confirmation. The reported number control in Colony Forming Units per carrier for the test microorganism is: ***Staphylococcus aureus* 1.38 x 10^6.**

Note: There were no protocol changes.
Note: An invalid data occurred due to a wash numbers control failure, resulting in a repeat assay.

V. RESULTS

MRID Number	Organism	Exposure Time	Lot No.	CFU/Carrier Average Log_{10}	Percent Reduction	Carrier Population (Log_{10} CFU/Carrier)
496845-13	*K. pneumoniae* (ATCC 4352)	15 minutes	Batch 2037-054	3.05	>99.9%	6.63
			Batch 2037-055	<1.47	>99.9%	
			Batch 2037-056	2.12	>99.9%	
496845-14	*S. aureus* (ATCC 6538)	16 minutes	Batch 2037-054	<1.48	>99.9%	6.14
			Batch 2037-055	1.26	>99.9%	
			Batch 2037-056	1.80	>99.9%	

VI. CONCLUSION

1. The submitted efficacy studies **support** the use of the product, White, as a laundry sanitizer with bactericidal activity against the following microorganisms on soft surfaces in the absence of organic soil with a 1:173 dilution in 200 ppm hard water.

    MRID 49684513 *Klebsiella pneumonia*, ATCC 4352, for a 15 minute contact time
    MRID 49684514 *Staphylococcus aureus*, ATCC 6538, for a 16 minute contact time

    Killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms on the tested lots.

VII. LABEL RECOMMENDATIONS

1. The proposed label claims are acceptable regarding the use of the product, White, as a laundry sanitizer for household application with bactericidal activity against *Klebsiella pneumonia* and *Staphylococcus aureus* for a 16-minute sanitization and for use on soft surfaces in the absence of organic soil and diluted as followed to produce a dilution factor of up to 1:173:

    - For High Efficiency (or front load) machines: 100 mL into the rinse cycle (17 Liters of up to 200 ppm of water at 20°C)
    - For Standard (or top load) machines: 150 mL into the rinse cycle (25.5 Liters of up to 200 ppm of water at 20°C)

    These claims **are supported** by the applicant's data.

2. Label must specify that the product is intended for household use.

3. On page 2 of the proposed label, registrant must remove the claim "Perfect dose every time". This type of language is misleading to the user.

4. On page 2 of the proposed label, registrant must remove "wash" from the claim "Just add to (wash) (rinse cycle) for hygienically clean laundry". Product was only tested using the dilution ratio as intended in the rinse cycle.

5. On the proposed label, registrant must remove "...kills 99.9% of bacteria (even below 30°C)...", "whatever temperature you wash at", and "Works (at) (in) (low temperatures) (cold water)" from the claims. Product is not proven effective in temperatures below 20°C.

6. Registrant must remove the phrase, "...that could cause skin infections," from the two claims on page 3 of the proposed label.

7. On page 3 of the proposed label, registrant must remove the claim "to stop the transmission from (clothing to clothing) (garment to garment)".

8. On page 4 of the proposed label, registrant must remove the claim "Virtually bacteria free freshness".